

1. An enzyme variant comprising a catalytic core domain exhibiting cellulolytic activity, which variant is derived from a naturally occurring parental cellulase by amino acid residue substitution, insertion or deletion or any combination thereof, and

- 20 2. The variant according to claim 1, which at position 119
(cellulase numbering) holds an amino acid residue selected from
the group consisting of histidine (H), aspartic acid (D),
asparagine (N), glutamine (Q), arginine (R), and phenylalanine
(F).

30 4. The variant according to claim 1, which at position 7
(cellulase numbering) holds an amino acid residue selected from
the group consisting of arginine (R), leucine (L), isoleucine
(I), tryptophan (W), and lysine (K).

35 5. A cellulase variant, which variant holds 4 or more of the following disulfide bridges: C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering).

6. The cellulase variant according to claim 5, which variant holds 5 or more of the following disulfide bridges: C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering).

7. The cellulase variant according to claim 5, which variant holds 6 or more of the following disulfide bridges: C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering).

8. The cellulase variant according to claim 5, in which variant a cysteine residue has been replaced by a different natural amino acid residue at one or more of the positions 16, 86, 87, 89, 189, and/or 199 (cellulase numbering).

9. The cellulase variant according to claim 5 selected from the group consisting the following variants derived from *Humicola insolens* endoglucanase V (EGV): C12G/C47M, C47G, C87M/C199G and C16M/C86G.

10. A method of reducing the thermostability of a cellulase comprising removal, by amino acid substitution, deletion or insertion, of one or more disulfide bridges selected from the group consisting of C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering).

11. A cellulase variant derived from a parental cellulase by substitution, insertion and/or deletion at one or more amino acid residues located in the substrate binding cleft at a position within an enzyme-substrate interactive distance from the substrate.

12. The cellulase variant according to claim 11, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more amino acid residues located in the substrate binding cleft at a distance of not more than 5 Å from the substrate.

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13. The cellulase variant according to claim 12, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following

5 positions: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 21a, 42, 44, 45, 47, 48, 49, 49a, 49b, 74, 82, 95j, 110, 111, 112, 113, 114, 115, 116, 119, 121, 123, 127, 128, 129, 130, 131, 132, 132a, 133, 145, 146, 147, 148, 149, 150b, 178, and/or 179 (cellulase numbering).

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14. The cellulase variant according to claim 12, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following

15 positions: 4, 5, 13, 14, 15, 16, 19, 20, 21, 21a, 42, 44, 47, 48, 49, 49a, 49b, 74, 82, 95j, 110, 111, 113, 115, 116, 119, 123, 129, 131, 132a, 133, 145, 146, 150b, 178, and/or 179 (cellulase numbering).

15. The cellulase variant according to claim 13 selected from
20 the group consisting the following variants derived from *Humicola insolens* endoglucanase V (EGV): T6S, R7I, R7W, Y8F, W9F, C12M/C47G, W18Y, W18F, S45T, S45N, D114N, F132D, Y147D, Y147C, Y147W, Y147V, Y147R, Y147G, Y147Q, Y147N, Y147K, Y147H, Y147F and Y147S.

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16. The cellulase variant according to claim 13 selected from the group consisting the following variants derived from *Humicola insolens* endoglucanase V (EGV): R4H, R4Q, K13L, K13R,

K13Q, P14A, P14T, S15T, S15H, C16M/C86G, A19P, A19T, A19G,
30 A19S, K20G, D42Y, D42W, C47G, E48D, E48Q, E48D/P49*, E48N/P49*, S110N, L115I, G116D, H119R, H119Q, H119F, N123A, N123M, N123Q, N123Y, N123D, V129L, D133N and D178N.

35 17. The cellulase variant according to claim 11, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more amino acid residues

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located in the substrate binding cleft at a distance of not more than 3 Å from the substrate.

18. The cellulase variant according to claim 17, which variant
5 has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 6, 7, 8, 10, 12, 13, 14, 15, 18, 20, 21, 45, 48, 74, 110, 111, 112, 113, 114, 115, 119, 121, 127, 128, 129, 130, 131, 132, 132a, 146, 147, 148, 150b, 178, and/or 179 (cellulase
10 numbering).

19. The cellulase variant according to claim 17, which variant
has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following
15 positions: 13, 14, 15, 20, 21, 48, 74, 110, 111, 113, 115, 119, 129, 131, 146, 150b, 178, and/or 179 (cellulase numbering).

20. A cellulase variant, in which variant an amino acid residue
has been changed into a conserved amino acid residue at one or
20 more positions according to Table 1, at which position(s) between 7 and 10 amino acid residues of the 11 residues identified in Table 1, are identical.

21. The cellulase variant according to claim 20, which variant
25 has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 13, 14, 15, 20, 21, 22, 24, 28, 32, 34, 45, 48, 50, 53, 54, 62, 63, 64, 65, 66, 68, 69, 70, 71, 72, 73, 74, 75, 79, 85, 88, 90, 92, 93, 95, 96, 97, 98, 99, 104, 106, 110, 111,
30 113, 115, 116, 118, 119, 131, 134, 138, 140, 146, 152, 153, 163, 166, 169, 170, 171, 172, 173, 174, 174, 177, 178, 179, 180, 193, 196, and/or 197 (cellulase numbering).

22. A cellulase variant according to claim 21 comprising one or
35 more of the following mutations (cellulase numbering):

K13L or L13K;

P14A or A14P;

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A74F or F74A;

T75V, T75A, T75G, V75T, A75T, G75T, V75A, V75G, A75V,
G75V, A75G, or G75A;

G79T or T79G;

W85T or T85W;

5 A88Q, A88G, A88R, Q88A, G88A, R88A, Q88G, Q88R, G88Q,
R88Q, G88R, or R88G;

Y90For F90Y;

L92A or A92L;

T93Q, T93E, Q93T, E93T, Q93E, or E93Q;

10 T95E or E95T;

S96T or T96S;

G97T, G97A, T97G, A97G, T97A, or A97T;

P98A or A98P;

V99L or L99V;

15 M104L or L104M;

V106F or F106V;

S110N or N110S;

T111I, T111V, I111T, V111T, I111V, or V111I;

G113Y or Y113G;

20 L115V or V115L;

G116S, G116Q, S116G, Q116G, S116Q, or Q116S;

N118T, N118G, N118Q, T118N, G118N, Q118N, T118G,

T118Q, G118T, Q118T, G118Q, or Q118G;

H119Q, H119N, Q119H, or N119H;

25 V129L or L129V;

I131L, I131A, L131I, A131I, L131A, or A131L;

G134A or A134G;

Q138E or E138Q;

G140N or N140G;

30 R146Q or Q146R;

S152D or D152S;

R153K, R153L, R153A, K153R, L153R, A153R, K153L,
K153A, L153K, A153K, L153A, or A153L;

L163V, L163W, V163L, W163L, V163W, or W163V;

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in position 38 holds F, I, L, or Q;
in position 42 holds D, G, T, N, S, K, or *;
in position 44 holds K, V, R, Q, G, or P ;
in position 45 holds N, or S;
5 in position 46 holds G, or S;
in position 47 holds C, or Q;
in position 48 holds D, E, N, or S;
in position 49 holds P, S, A, G, or *;
in position 49a holds C, or *;
10 in position 49b holds N, or *;
in position 50 holds G, or N;
in position 53 holds A, G, K, or S;
in position 54 holds F, or Y;
in position 62 holds F, or W;
15 in position 63 holds A, or D;
in position 64 holds D, I, or V;
in position 65 holds D, E, N, or S;
in position 68 holds D, N, P, or T;
in position 69 holds A, S, or T;
20 in position 70 holds L, or Y;
in position 71 holds A, or G;
in position 72 holds F, W, or Y;
in position 73 holds A, or G;
in position 74 holds A, or F;
25 in position 75 holds A, G, T, or V;
in position 79 holds G, or T;
in position 82 holds E, or *;
in position 88 holds A, G, Q, or R;
in position 90 holds F, or Y;
30 in position 92 holds A, or L;
in position 93 holds E, Q, or T;
in position 95 holds E, or T;
in position 95j holds P, or *;
in position 96 holds S, or T;

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in position 97 holds A, G, or T;
in position 98 holds A, or P;
in position 99 holds L, or V;
in position 104 holds L, or M;
5 in position 106 holds F, or V;
in position 110 holds N, or S;
in position 111 holds I, T, or V;
in position 113 holds G, or Y;
in position 115 holds L, or V;
10 in position 116 holds G, Q, or S;
in position 118 holds G, N, Q, or T;
in position 119 holds H, N, or Q;
in position 129 holds L, or V;
in position 131 holds A, I, or L;
15 in position 132 holds A, P, or T;
in position 133 holds D, K, N, or Q;
in position 134 holds A, or G;
in position 138 holds E, or Q;
in position 145 holds A, D, N, or Q;
20 in position 146 holds Q, or R;
in position 150b holds A, or *;
in position 152 holds D, or S;
in position 153 holds A, K, L, or R;
in position 163 holds L, V, or W;
25 in position 166 holds G, or S;
in position 169 holds F, or W;
in position 170 holds F, or R;
in position 171 holds A, F, or Y;
in position 172 holds D, E, or S;
30 in position 173 holds E, or W;
in position 174 holds F, M, or W;
in position 177 holds A, or N;
in position 178 holds D, or P;
in position 179 holds N, or V;

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24. A cellulase variant having an altered anion tenside sensitivity, and which variant is from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 2, 4, 7, 8, 10, 13, 15, 19, 20, 21, 25, 26, 29, 32, 33, 34, 35, 37, 40, 42, 42a, 43, 44, 48, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 70, 72, 76, 79, 80, 82, 84, 86, 88, 90, 91, 93, 95, 95d, 95h, 95j, 97, 100, 101, 102, 103, 113, 114, 117, 119, 121, 133, 136, 137, 138, 139, 140a, 141, 143a, 145, 146, 147, 150e, 150j, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160c, 160e, 160k, 161, 162, 164, 165, 168, 170, 171, 172, 173, 175, 176, 178, 181, 183, 184, 185, 186, 188, 191, 192, 195, 196, 200, and/or 201 (cellulase numbering).

25. A cellulase variant, in which variant an amino acid residue
20 has been substituted at one or more of the following positions:
17, 85, 86 87, 88, and/or 89 (cellulase numbering).

26. A *Humicola insolens* EGV variant, in which one or more of the following mutations have been introduced: D42W, D42Y, or L70Y.

27. A *Thielavia terrestris* cellulase variant, in which variant one or more of the following mutations have been introduced: P19A, G20K, Q44K, N48E, Q119H or Q146 R.

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28. The *Thielavia terrestris*/Q119H variant.

29. A *Pseudomonas fluorescens* cellulase variant, in which variant one or more of the following mutations have been introduced: Y4R, H15S, N119Q or Q146R.

30. A *Crinipellis scabellia* cellulase variant, in which one or more of the following mutations have been introduced: V4R, T132a*, Q133D or Q146R.

31. A method for improving the properties of a cellulolytic enzyme by amino acid substitution, deletion or insertion, the method comprising the steps of:

a. constructing a multiple alignment of at least two amino acid sequences known to have three-dimensional structures similar to endoglucanase V (EGV) from *Hemicola insolens* known from Protein Data Bank entry 4ENG;

b. constructing a homology-built three-dimensional structure of the cellulolytic enzyme based on the structure of the EGV;

c. identifying amino acid residue positions present in a distance from the substrate binding cleft of not more than 5Å;

d. identifying surface-exposed amino acid residues of the enzyme;

e. identifying all charged or potentially charged amino acid residue positions of the enzyme;

f. choosing one or more positions wherein the amino acid residue is to be substituted, deleted or where an insertion is to be provided; and

g. carrying out the substitution, deletion or insertion by using conventional protein engineering techniques.

32. The method according to claim 31, wherein step f. is carried out by choosing positions which, as a result of the alignment of step a., carry the same amino acid residue in a majority of the aligned sequences.

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33. The method according to claim 32, wherein step f is carried out by choosing positions which, in the aligned sequences, carries different amino acid residues.

34. A method of improving the specific activity of a naturally occurring parental cellulase by carrying out a substitution, deletion or insertion at amino acid residue positions present in a distance from the substrate binding cleft of not more than 5Å.

35. A method of altering the pH activity profile, the pH activity optimum, the pH stability profile, or the pH stability optimum of a naturally occurring parental cellulase by altering the electrostatic environment either locally or globally by carrying out a substitution, deletion or insertion at amino acid residue positions present either in a distance from the substrate binding cleft of not more than 5Å, or at surface-exposed amino acid residue positions of the enzyme; preferably by a substitution involving a charged or potentially charged residue, this residue either being the original residue or the replacement residue.

36. A method of altering the stability of a naturally occurring parental cellulase in the presence of an anionic tenside or anionic detergent component by altering the electrostatic environment either locally or globally by carrying out a substitution, deletion or insertion at one or more surface-exposed amino acid residue positions of the enzyme.

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